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(54) Title of the Invention: DEVICE FOR DETERMINING THE BASE SEQUENCES OF NUCLEIC ACIDS

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Specification
Title of the Invention: DEVICE FOR DETERMINING THE BASE SEQUENCES OF NUCLEIC ACIDS

Range of Patent Claims

1. A device for determining the base sequences of nucleic acids equipped with the following features. It has a tank into which fragments of nucleic acids that were cleaved to various lengths at the position of each base are injected, a means for causing the aforementioned fragments to move across the tank described above at speeds corresponding to their molecular weight and the means for detecting the aforementioned fragments that are mounted in the path of motion.
2. The device for determining the base sequences of nucleic acids described in Claim 1 of the Range of Patent Claims with the following features. The tank described above is an electrophoresis tank and the means for inducing motion described above is an electrophoretic drive power supply that applies an electrophoretic force to the aforementioned fragments.
3. The device for determining the base sequences of nucleic acids described in Claim 1 of the Range of Patent Claims with the following features. The fragments described above are labeled with a radioactive substance and the aforementioned detection means is a radioactivity detector.
4. The device for determining the base sequences of nucleic acids described in Claim 1 of the Range of Patent Claims with the following features. The fragments described above are labeled with a fluorescent substance and the aforementioned detection means is a light detector.

5. The device for determining the base sequences of nucleic acids described in Claim 1 of the Range of Patent Claims with the following features. The device is equipped with at least four of the aforementioned detection means.
6. The device for determining the base sequences of nucleic acids described in Claim 1 of the Range of Patent Claims with the following features. Each of the detection means described above detects several types of the fragments described above.
7. A device for determining the base sequences of nucleic acids equipped with the following features. It has a tank into which fragments of nucleic acids that were cleaved to various lengths at the position of each base are injected, a means for causing the aforementioned fragments to move across the tank described above at speeds corresponding to their molecular weight, the means for detecting the aforementioned fragments that are mounted in the path of motion and a means for deciphering the base sequences of the fragments based on the detection signals from the aforementioned detection means.
8. The device for determining the base sequences of nucleic acids described in Claim 7 of the Range of Patent Claims with the following features. The tank described above is an electrophoresis tank and the means for inducing motion described above is a electrophoretic drive power supply that applies an electrophoretic force to

the aforementioned fragments.

9. The device for determining the base sequences of nucleic acids described in Claim 7 of the Range of Patent Claims with the following features. The fragments described above are labeled with a radioactive substance and the aforementioned detection means is a radioactivity detector.
10. The device for determining the base sequences of nucleic acids described in Claim 7 of the Range of Patent Claims with the following features. The fragments described above are labeled with a fluorescent substance and the aforementioned detection means is a light detector.
11. The device for determining the base sequences of nucleic acids described in Claim 7 of the Range of Patent Claims with the following features. The device is equipped with at least four of the aforementioned detection means.
12. The device for determining the base sequences of nucleic acids described in Claim 7 of the Range of Patent Claims with the following features. Each of the detection means described above detects several types of the fragments described above.

Detailed Description of the Invention

Fields of Use of the Invention

This invention pertains to devices that determine the base sequences in nucleic acids such as DNA (dioxyribonucleic acids) or RNA (ribonucleic acids).

Background of the Invention

Advances in genetic engineering have made it necessary to decipher the genetic information contained in DNA and RNA quickly. In DNA, for example, four types of bases (adenine (A), guanine (G), cytosine (C) and thymine (T)) are arranged and the genetic information is determined by the sequencing of these elements. Intense research is being conducted into the relationship between the phenotypic expression in organisms and the base sequences along the DNA. For this reason, a means for determining the base sequences of the DNA is necessary and several methods of making such determinations have been proposed to date. ("Cellular Engineering" Vol. 1, No. 1, No. 2 (1982))

Among the methods proposed, the most widely used method for determining DNA base sequences is the Maxam-Gilbert Method described below. In this method, the following steps are used to make the determination.

(1) First the DNA fragment for which the sequence is to be determined is separated.
(2) Both ends of the separated DNA fragment are labeled with a radioactive phosphorous (^{32}P).
(3) A double-stranded DNA fragment that was labeled on both ends was separated and a single strand DNA fragment with a ^{32}P label on just one end was prepared. Alternatively, DNA fragments with a ^{32}P label on just one end were also prepared by using a restricting enzyme to cleave a DNA fragment that was labeled on both ends and then separating the DNA fragments.
(4) A modified chemical substance that had a modifying effect on the base G was used on the DNA fragment that had been labeled on one end using ^{32}P and then a cleaving chemical substance was used to cleave at the modified base G position. The cleaving was performed so that there would be an average of one occurrence per DNA fragment. As shown in Figure 1(b), doing so produced DNA fragments of varying lengths that had ^{32}P labels on one end and had been cleaved at the base G position on the other end. In this case, as shown in Figure 1(c), this also produced DNA fragments that did not have a ^{32}P label on one end, but as described below, the radiation from the ^{32}P was measured, so those fragments were not a problem.
(5) The same sort of process was carried out individually for the bases A, C and T.

Note also that there is no suitable chemical substance that acts specifically on the positions of the bases A and T. For this reason, the cleaving at the base A position was carried out, for example, using a chemical substance that acted on both bases G and A. The cleaving process (G + A) was performed at the base G position and the base A position. DNA that had been cleaved at the base G position that had no pattern was taken to have been a DNA fragment that had been cleaved at the base A position. In the same way, when cleaving at the base T position, a chemical substance that acted on both bases C and T would be used and a cleaving process (C + T) would be performed at the base C position and the base T position. If the DNA fragment that had been cleaved at the base C position had no pattern, it was taken to be a DNA fragment that had been cleaved at the base T position. In this way, one end was labeled using ^{32}P and the other end was cleaved at the base G, G + A, C + T

or C positions, producing groups of four types of DNA fragments.

(6) These DNA fragments were lined up on an electrophoresis plate (not shown in the diagram) by type and underwent electrophoresis simultaneously.

(7) After running the electrophoresis for a suitable length of time, the gel was affixed to a dry photographic plate, which was exposed to the ^{32}P radiation. Patterns were obtained of 4 bands corresponding to the fragments that had been cleaved at the bases G, G + A, C + T and C on the dry photographic plates. The electrophoresis speed of the DNA fragments varied according to their base count. The smaller and shorter the base count, the faster the electrophoresis, so such samples move quickly from one end to the other.

(8) Lastly, the base sequences on the DNA from the side with the ^{32}P-labels can be determined sequentially by reading in the fragments that were cleaved at the bases G, G + A, C + T and C in order from shortest to longest. Problems with this method include the need for the use of radioactive phosphorous, the length of time it takes to expose the dry photographic plates (approximately one day and one night, although the electrophoresis can be finished in a few hours) and the inconvenience of only being able to make determinations for approximately 200 to 300 bases at a time.

Purpose of the Invention

Based on the above problems, the purpose of this invention is to provide a device which can determine the base sequences quickly and easily.

Outline of the Invention

The features that are needed in a device that determines the base sequences of nucleic acid in order to achieve the above objectives are a tank for injecting the nucleic acid fragments that have been cleaved to various lengths at each of the base positions, a means for making the nucleic acid fragments moved within the aforementioned tank at a speed corresponding to their molecular weight and a means for detecting the aforementioned nucleic acid fragments which has been mounted in the paths of motion of the aforementioned nucleic acids.

The other features of this invention include(?)a tank for injecting the nucleic acid fragments that have been cleaved to various lengths at each of the base positions, a means for making the nucleic acid fragments moved within the aforementioned tank at a speed corresponding to their molecular weight, a means for detecting the aforementioned nucleic acid fragments which has been mounted in the paths of motion of the aforementioned nucleic acids and a means for deciphering the base sequences of the fragments based on the detected signals from the aforementioned detection means.

Embodiments of the Invention

An embodiment of this invention is described below with reference to Figure 2 and Figure 3.

First, the DNA to be measured is divided into a number of fragments. The dividing up of this DNA is carried out using a restriction enzyme that can recognize and cleave particular base sequence positions along the DNA. Among these restriction enzymes are some which can recognize and cleave several base sequences (4, 6, etc.). The fragments that are formed this way were numbered F1, F2, ... Fn. The problem is to determine the base sequences of these DNA fragments.

Next, after separating and purifying each DNA fragment, the fragments are processed according to the conventional methods described above, resulting in DNA fragments that have been labeled with ^{32}P or a fluorescent substance on one end.

After that, the 4 types of bases G, G + A, C + T and C are modified using a chemical substance that either specifically or selectively modifies them. They are then partially cleaved using a chemical substance that cleaves the modified positions selectively.

Of the above fragments F_1, those that had the base G position cleaved were called F_{1G}. Among those F_{1G} fragments were some that had one end labeled and the other end cleaved at various base G positions, those on which both ends had been cleaved at various base G positions as well as fragments that contained no labels. Of these, if we focus only on those that have been labeled on one end, then we can set the fragments in order of size based on where the various base G position cleavage was. We were able to set the fragments in order of size for the bases G + A, C + T and C as well.

Next, these four types of fragments (F_{1G}, F_{1G+A}, F_{1C+T} and F_{1C}) were injected into the electrophoresis tank and separated. The phoresis speed of the each of the fragments varied according to their length, which is how they were separated. Those fragments that were the same length underwent phoresis at the same speed. With

conventional methods, the bands would be transferred to dry photographic plates after sufficient electrophoresis and the base sequences would be determined by analyzing those patterns. With this invention, the radioactive bands or the bands that have been labeled using a fluorescent substance are detected at a particular point along the phoresis path while the phoresis is underway.

Figure 2 shows a device for determining base sequences that has been equipped with an output device, a data processing device and detectors of this invention as well as an electrophoresis tank. On either end of the electrophoresis tank 1 are the positive and negative electrodes 2A and 2B. There is an electrophoresis power supply 3 that runs an electric current between the two electrodes 2A and 2B. On the upper surface of the electrophoresis tank 1 is the separation plate 4. The detectors 5(a), 5(b), 5(c) and 5(d) are set up at 4 specific places along the separation plate 4 in the phoresis path of the four fragments F_{1G}, F_{1G+A}, F_{1C+T} and F_{1C} that were cleaved at the bases G, G + A, C + T and C. The number of detectors need not be restricted to 4 and there could be times when 5 are used, in which case 1 of them would be used as a reference. Again, the 4 could be taken together as a set and several of them could be installed. They could also be installed in sets of 5. These detectors are radiation detectors when the label used on one end of the DNA fragment is ^{32}P and when the label is a fluorescent substance, they are light detectors. Note also that a single detector could also be used if the strength of the radiation or the type of light were changed so that each type of fragment could be identified. In this way, a single detector could be used to detect several types of fragments as they passed by. These detectors 5(a), 5(b), 5(c) and 5(d) detect each of the fragments as they pass through during electrophoresis and they output detection signals. We will describe the state of those signals using Figure 3.

In Figure 3, the signals from each of the detectors 5(a), 5(b), 5(c) and 5(d) in the path of the phoresis of each of the fragments F_{1G}, F_{1G+A}, F_{1C+T} and F_{1C} are amplified in the amplifier 6. The amplified signal is sent out as the sort of signal shown in Figure 3(b) from the amplifier 6 with the passage of time. The peak portion of the signal strength indicates that the detectors 5(a), 5(b), 5(c) and 5(d) have detected the phoresis of the fragments of the bases G, G + A, C + T and C.

Next, this amplified signal is input into the data processing device 7 shown in Figure 2. There, the signal is deciphered and the base sequence is determined. Of the signals shown in Figure 3(b), the peaks located in the lower half of the diagram indicate fragments that underwent phoresis quickly and in a shorter amount of time. This means that DNA fragments that were shortest and had the lowest molecular weight were detected. The detection time was different for each time the length changed by one base, which makes it possible to determine the base sequences by reading sequentially, beginning with the peaks that appear in the shortest amount of time. For example, the signals shown in Figure 3(b), the signal from the F_{1G} fragment line appears first, so the terminal is base G. Subsequently, the sequences for bases A, G, C, A, T and C were determined. After the output for these has been organized in the data processing device 7, the letters are output in the sequence order. The output device 8, for example has output GAGCATC on the printer.

Note also that the device that determines the base sequences of the nucleic acids could be anything that is equipped with a detection means. Other devices that can be installed include the amplifier mentioned above, the data processing device and the output device.

Effect of the Invention

This invention has the following sorts of effects.

(1) There is no need to take photographs of the phoresis patterns as is done conventionally. Determinations of the base sequences can be made in a short period of time while the phoresis is still underway.
(2) With conventional methods, extreme care was required for the time necessary to the electrophoresis. That is, if the time wasn't long enough, there wouldn't be sufficient separation of DNA fragments so only 100 bases could be read at most. If too much time was taken, the smaller DNA fragments would reach the end of the gel and become unreadable. That required taking the time to divide the phoresis into several stages. This invention allows the measurement of everything from small fragments to those that are so large they challenge the limit of the separation capacity of the gel.
(3) The above arguments could also be made for RNA.

Brief Descriptions of the Drawings

Figure 1(a) is a diagram showing a model of a DNA fragment with one end labeled. Figure 1(b) shows

a model of the fragment in Figure 1(a) that underwent a base G-specific reaction that had then undergone further cleavage at the base G position. Figure 1(c) is a diagram showing a fragment without the DNA fragment shown in Figure 1(b). Figure 2 is a diagram showing an embodiment of this invention. Figure 3 is a diagram showing the signal output from the detector and the amplifier.

1: Electrophoresis Tank, 2A And 2B: Electrodes, 3: Electrophoresis Power Supply, 4: Separation Plate, 5(A), 5(B), 5(C) And 5(D): Detectors, 6: Amplifier, 7: Data Processing Device, 8: Output Device.

Representing Patent Attorney: Akio TAKAHASHI [illegible stamp]

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Figure 1

Figure 2

5(a) 5(b) 5(c) 5(d)

Figure 3

Time

Signal Strength

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